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CHARACTERIZATION OF RAMBUTAN (*Nephelium lappaceum* L.) SEED FAT AND ITS MIXTURE WITH COCOA BUTTER FOR POTENTIAL APPLICATION IN DARK CHOCOLATE

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by

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LIST OF SYMBOLS AND ABBREVIATION

%	Percentage
°C	Celsius
µg	Microgram
a*	Redness
A.V	Acid value
ANOVA	Analysis Of Variance
AOAC	Association Of Official Analytical Chemists
b*	Yellowness
CB	Cocoa butter
DSC	Differential scanning calorimeter
DPPH	1,1-Diphenyl-2-Picrylhydrazyl
RSF	Rambutan seed fat
SFC	Solid fat content
g	Gram
GC	Gas Chromatography
GC-MS	Gas Chromatography–Mass Spectrometry
HPLC	High Performance Liquid Chromatography

TAG	Triacylglycerl
Kg	Kilogram
TGA	Thermal gravimetric analysis
L*	Lightness
meq	Milliequivalent
mg	Milligram
min	Minute
mL	Milliliter
mg/L	per milligram liter
MUFA	Monounsaturated Fatty Acid
nm	Nanometer
p-AnV	P-Anisidine Value
TPC	Total phenolic compound
P.V	Peroxide Value
PUFA	Polyunsaturated Fatty Acid
ppm	parts-per-million
PLM	Polarized light microscopy
XRD	X-ray diffraction

I.V	Iodine value
FFA	Free fatty acid
SFA	Saturated Fatty Acid
TG	Triglycerides
SPSS	Statistical Package For Social Science
TOTOX	Total oxidation
PAV	P-anisidine value
UK	United Kingdom
US	United State
Choc Cb	Dark chocolate made from CB
Choc M1	Dark chocolate made from M1
Å	Angstrom

PENCIRIAN LEMAK BIJI RAMBUTAN (*NEPHELIUM LAPPACEUM L.*) DAN CAMPURAN BERSAMA MENTEGA KOKO UNTUK POTENSI APLIKASINYA DALAM PEMBUATAN COKLAT GELAP

ABSTRAK

Biji rambutan adalah salah satu produk sampingan yang mempunyai potensi untuk dimanfaatkan, terutamanya lemak biji rambutan (RSF) yang sebahagiannya boleh dicampurkan ke dalam mentega koko (CB). Dalam kajian ini, lemak daripada biji rambutan yang telah ditapai selama enam hari dan dipanggang dicampur dengan nisbah yang berbeza dengan mentega koko, iaitu 100/0, 80/20, 60/40, 40/60, 20/80, dan 0/100 (w / w) CB kepada RSF, dilabel sebagai CB, M1, M2, M3, M4 dan RSF. Perubahan terhadap sifat-sifat fizikal dan kestabilan haba telah dikaji. Kajian mendapati bahawa campuran tertentu CB dan RSF, seperti M1 (80% CB + 20% RSF) menunjukkan puncak maksimum sewaktu perlakuan haba, kandungan lemak pepejal (SFC), morfologi, dan polymorfisme yang sama dengan CB. Tambahan pula, corak XRD menunjukkan bahawa M1 mempunyai jarak pendek yang sama dan pantulan sudut lebar seperti CB pada suhu 20 °C. Di samping itu, sampel CB dan M1 mempunyai kestabilan terma dan kekerasan terma yang lebih tinggi daripada campuran lain. Manakala kesan suhu berbeza terhadap kelikatan sampel CB dan M1, di mana CB dan M1 mempunyai kelikatan yang lebih rendah daripada campuran lain seiring dengan peningkatan suhu. Kajian ini juga dijalankan untuk mengkaji analisis kimia seperti komposisi asid lemak, nilai asid, asid lemak bebas, nilai iodin, aktiviti antioksidan, komposisi trigliserida dan pengoksidaan lemak. Hasil kajian menunjukkan bahawa asid laurik, asid palmitik, asid lemak stearik di dalam lemak rambutan kurang daripada kandursan di dalam mentega koko, tetapi asid oleik adalah yang tertinggi dalam RSF. Walau bagaimanapun, campuran CB (100% CB + 0% RSF) dan M1 (80% CB + 20% RSF) menunjukkan aktiviti antioksidan yang paling tinggi dan komposisi trigliserida biasa seperti Gliserol-1, 3-dipalmitate-2-oleate (POP), gliserol-1-palmitat-2-oleat-3-stearat (POS) dan gliserol-1,3-distearat-2-oleat (SOS). Kajian ini juga mendapati bahawa sifat-sifat nilai tambah seperti rasa Pyrazine diperhatikan tertinggi dalam sampel RSF (0% CB + 100% RSF) dirbanding kan dengan campuran lain. Sebaliknya, coklat gelap yang dihasilkan dengan M1 (Choc M1) menunjukkan penumpuan yang hebat dibandingkan dengan coklat gelap yang dihasilkan dengan CB (Choc CB) dari segi warna, kelikatan, profil tekstur dan perlakuan haba. Dalam penilaian deria, sifat kedua-dua coklat gelap adalah sedikit berbeza, tetapi tidak terdapat perbezaan yang signifikan di antara sampel coklat gelap. Oleh itu, Choc M1 menunjukkan penerimaan keseluruhan yang lebih tinggi sedikit di kalangan ahli panel berbanding Choc CB. Berdasarkan keputusan, kajian menunjukkan bahawa bahagian campuran 80% lemak koko + 20% lemak biji rambutan boleh digunakan sebagai alternatif kepada mentega koko, manakala campuran yang lebih tinggi (lebih daripada 20% RSF) mengubah keseluruhan sifat-sifat asal mentega koko. Potensi RSF yang akan digunakan sebagai alternatif mentega koko, dan kemungkinan aplikasi dalam pelbagai industri termasuk pembuatan coklat.

CHARACTERIZATION OF RAMBUTAN (*Nepheliumlappaceum* L.) SEED FAT AND ITS MIXTURE WITH COCOA BUTTER FOR POTENTIAL APPLICATION IN DARK CHOCOLATE

ABSTRACT

Rambutan seed is one of rambutan by-product that has a potential to be utilized, especially as rambutan seed fat (RSF) and these fats can be partially incorporated into cocoa butter (CB). In this study, the fat from six days fermented and roasted rambutan seed was mixed with different proportions of cocoa butter, namely, 100/0, 80/20, 60/40, 40/60, 20/80, and 0/100 (w/w) CB to RSF, as CB, M1, M2, M3, M4 and RSF respectively. The changes that occurred to the physical properties and thermal stability were investigated. The results suggested that certain mixtures of CB and RSF, such as M1 (80%CB+20%RSF) exhibited the peak maxirnum during thermal behavior, polymorphism, morphology, and solid fat content of M1 sample similar to that of CB. Furthermore, the X-ray diffraction patterns (XRD) showed that M1 had the same short spacing and wide angle reflections as those of CB at a temperature of 20 °C. In addition, CB and M1 sample had higher thermal stability and hardness index than other mixtures. The effect of different temperatures on the viscosity of CB and M1 sample showed CB and M1 had a lower viscosity than other mixtures with increasing temperature. This study was determined the chemical analysis such as fatty acid composition, acid value, free fatty acid, iodine value, antioxidant activity, triglycerides composition and lipid oxidation. The results showed that lauric acid, palmitic acid, and stearic fatty acid in rambutan fat were less than that in cocoa butter, whereas the oleic acid was the highest in RSF. The CB and M1 showed the highest antioxidant activity and the

typical triglycerides compositions such as, Glycerol-1, 3-dipalmitate-2-oleate (POP), glycerol-1-palmitate-2-oleate-3-stearate (POS) and glycerol-1,3-distearate-2-oleate (SOS). The study also found that value-added properties such as desirable pyrazine flavor were observed the highest in the RSF sample (0%CB+100%RSF) in comparison with other mixtures. On the other hand, the dark chocolate made from M1 (Choc M1) showed similar to dark chocolate made from CB (Choc CB) in color, viscosity, texture profile and thermal behavior. In sensory evaluation, the attributes of two dark chocolate were showed no significant differences between the dark chocolate samples. Thus, the ChocM1 showed no significant differences in overall acceptability between panelists than ChocCB. Based on the results, the study showed that mixture proportion, 80% cocoa butter + 20% rambutan seed fat can be used as a cocoa butter alternative, whereas a higher proportion (more than 20% RSF) completely altered original cocoa butter properties. The RSF potential to be utilized as cocoa butter alternatives and possibilities of application in different branches of industries include chocolate manufacturing.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background information

Cocoa butter is a main component in the manufacture of all kinds of chocolate. Chocolates are commonly known as sweet, processed foods produced from the seeds of tropical fruit (Zzaman and Yang, 2013). Cocoa butter is therefore the exclusive phase in the persistent fat used in the manufacture of chocolate (Lanness *et al*., 2003). At present, the price of cocoa gradually increased as the price of cocoa was about 1590 US dollars per tonne in 2006, while the price in 2011 increased to about 3140 US dollars per tonne (ICCO, 2011). In addition, about 30% of the world's cocoa crops have been broken by plague and diseases as well as by environmental change. However, the fat content of the cocoa bean is small in amounts as compared to the other fatty crops about 50-58% of the cocoa beans. Less amount of fat seed content and cultivated in a few countries having a tropical climate, can be unstable and expensive (Knapp, 2007). Therefore, the most important economic and technological reasons, researchers have thought for the discovery of other fats as an alternative to cocoa butter in the chocolate industry (Dewettink and Depypere, 2011; Issara *et al*., 2014). Technological and economic aspects require that used other plant fats instead of cocoa butter in the confectionery industry (Jahurul *et al*., 2013).

Rambutan (*Nephelium lappaceum* L.) fruit is an exotic fruit that grows in Southeast Asia (Marisa, 2006). Rambutan is grown in Malaysia in large numbers and in vast areas and has a production rate of 80,000 tonne per year, while

Indonesia's production rate is about 148,000 tonne per year. Thailand was produced about 430,000 tonne per year and the Philippines about 20,000 tonne per year. The seeds of rambutan contain a high amount of fat, which is estimated at about 14% to 41% (Dadshani, 2002). Rambutan seed fat consists of relatively balanced saturated fatty acid (total ±50%, arachidic ±34%, palmitic ±6%, and stearic ±7%) and monounsaturated fatty acid (total ±48%, mostly oleic ±40%) that form a stable-solid appearance in room temperature, so that it's suitable to be used as continuous phase in the confectionery product. In addition, the fat of rambutan seeds is rich in ascorbic acid when modification subjected to including interesterification to improve the physicochemical properties of fat. It is also rich in zinc element by about 40.6 mg per 100 g and calcium component about 160 mg per 100 g, respectivelly (Dadshani, 2002). Rambutan seed fat contains sufficient amounts of minerals needed for human needs (Dietary Reference Intake (DRIs), 2001).

Fermentation and roasting treatments are processing methods that are usually carried out to improve the characteristics besides the eating palatability of food products. Fermentation can reduce undesirable and toxic elements present in food Afoakwa *et al.* (2012) and Camu *et al*. (2008) showed that the fermentation of fresh cocoa beans contributed to lower flavor content, as well as reduced levels of bitter taste in the product. Many applications of fermentation in particular agricultural products have been reported to lead in the production of high value-added and commercially high-priced functional food products (Couto & Sanromán, 2006).

In cocoa bean production, apart from improving the palatability, roasting process is used to induce Maillard reactions that generates the unique cocoa flavor. This step is very important in order to produce edible and high quality cocoa bean product (Bonvehi & Coll, 2002). There is a possibility of substituting cocoa butter and replacing it with rambutan seed oil (Gray, 2011). In a previous study, Febrianto (2013) found that the fermentation and roasting processes of rambutan seed provide the improvement of consistency, thermal characteristic, higher antioxidant activity, and develop the flavor in fat found in the seeds of rambutan. The six days fermentation of rambutan kernel and then roasting has been found to exhibit good fat properties in term of physicochemical and cocoa-like flavor development. These improvements proposed that fat from fermented and roasted rambutan seed is potential to be utilized as cocoa butter alternative. Meanwhile, the last research showed that rambutan seed fat can be used as an alternative to cocoa butter after mixing with certain proportions of cocoa butter and then applied in chocolate manufacturing (Zzaman *et al*., 2014a).

Chocolate considered a complex suspension in the continuous lipid phase (Fenandes *et al*., 2013). Cocoa can be defined as the non-greasy component of cocoa liquid (soft-grained cocoa beans), which is used in the chocolate-making industry (about 55% cocoa butter) or cocoa powder and accounts for approximately 12% of fat (Chan *et al.*, 1994). In addition, chocolate contains sugar, milk and minerals specifically potassium, magnesium, copper and iron. There are three types of chocolate such as dark, milk and white chocolate are differences in their components of fat, milk and cocoa butter and therefore differ in their components of carbohydrates, fat and protein. In most chocolate products, the fat content ranges from 25-35% in the final product. (Zzaman and Yang, 2013). Afoakwa *et al.* (2008b) mentioned that during the manufacture of chocolate, the component of cocoa butter and its crystallization play an important role in the quality of the final product. The crystalline state and the ratio of solid fat content are very important in determining the melting point of the chocolate product. Solís-Fuentes *et al.* (2010) spotted that the last peak curve of the rambutan seed fat-melting point was (~ 45^{o}C), showed higher than cocoa butter, and this is considered beneficial when making the manufacturing process. On the other hand, rambutan seed fat is much smoother than cocoa butter when it is low in temperature and is more solid and consistent at higher temperatures. This behavior is caused by a difference in composition between the cocoa butter and the fat of rambutan seed (Solís-Fuentes *et al.*, 2004; Lanes *et al.*, 2003). This thesis will discuss about the *rambutan* by-product, especially *rambutan* seeds, along with the application of solid-state fermentation and roasting process to produce *rambutan* seed fat. Hence, the aim of this study was to utilize of fat obtained from six days fermented and roasted *rambutan* seed fat sample mixed with cocoa butter in different proportions to study its physicochemical characteristics, flavor development and possible application in the dark chocolate manufacturing. This information will be a good input for an evaluation of the compatibility of *rambutan* seed fat with cocoa butter in producing dark chocolate using the best mixture which was more similar to cocoa butter properties.

1.2 Problem statements

1- The high and increase price of cocoa butter, low supply and high demand.

2- Few countries cultivated and supplier of cocoa butter.

3- Therefore, we seek to find a cheaper and more readily available alternative fat to cocoa butter in producing chocolates derived from natural source to increase product quality and reduce production costs, to the need of production process alteration and creation of new business values.

4- Rambutan seed is an industrial by-product considered as waste, utilization of rambutan seed fat is possible to produce value-added product.

1.3 Objectives

The main objective of this study was to characterize the physicochemical properties and flavor compounds of rambutan seed fat and mixed with cocoa butter for potential application in dark chocolate. The specific objectives of this study are as follows:

1- To determine the physical properties of rambutan seed fat and its mixtures with cocoa butter, such as color, texture (hardness), microstructure, thermal behavior, polymorphic behavior, solid fat content, thermal stability and viscosity.

2- To investigate the chemical analysis of rambutan seed fat and its mixture with cocoa butter, such as fatty acid, free fatty acid, acid value, antioxidant activity, triglycerides composition, lipid oxidation and iodine value.

3- To qualitative the flavor compound of rambutan seed fat and its mixture with cocoa butter, such as ester, carboxylic acid, hydrocarbon, pyrazine, aldehyde, alcohol and ketone compound.

4- To determine the physical properties, such as color, viscosity, thermal behaviour and texture properties (hardness), with sensory evaluation between dark chocolate made from various cocoa butter and rambutan seed fat mixture.

CHAPTER 2

LITERATURE REVIEW

2.1 Cocoa beans

2.1.1 Background of cocoa beans

The general name of cocoa is theobroma belongs to the sterculiaceae family. It has about 30-50 beans, covered with pulp as shown in Figure 2.1. About five centuries ago, the beginning of found of cocoa beans in Latin America, and within a few years, dominated and cultivated in Europe and then spread throughout the world (International Cocoa and Commodities Organization, ECO, 2000). Cocoa was inserted in Sri Lanka in 1798 and then spread to Singapore, Fiji in 1880, Queensland in 1886 and Ziljibar in 1887. In Malaysia, cocoa was inserted in 1778 and in Hawaii in 1831 and in India, the cocoa was inserted in the 20th century (Nair, 2010). Forastero, Criollo, and Trinitario are among the main types of cocoa. Although Forastero is one of the most common types of cocoa, its quality is not good (poor). Forastero affects 95% of the world's cocoa production. At the last time, the Forastero has been planted in large areas of Brazil and West Africa (Food and Agriculture Organization (FAO), 1977; Nair, 2010). The other type of cocoa is the Criollo and can be described as red or yellow pods mature and seeds are large in size. It is a kind of high quality and tasty seed when compared to the Forastero but the yield is low. The major shortage of Criollo variety is low content of cocoa fat compared with Forastero variety. Trinitario type is a combination of high quality and variety (mixture of Criollo and Forastero) (Yanamoto, 1995). Approximately 40 cocoa beans are enclosed in a pod (Figure 2.1) and it requires up to 6 months to obtain a mature pod. Generally, cocoa pods have 20 cm long and 15 cm wide. The pods are opened and the beans removed from their mucilage (white cover), beans contain around 55 % fat, and 45% moisture.

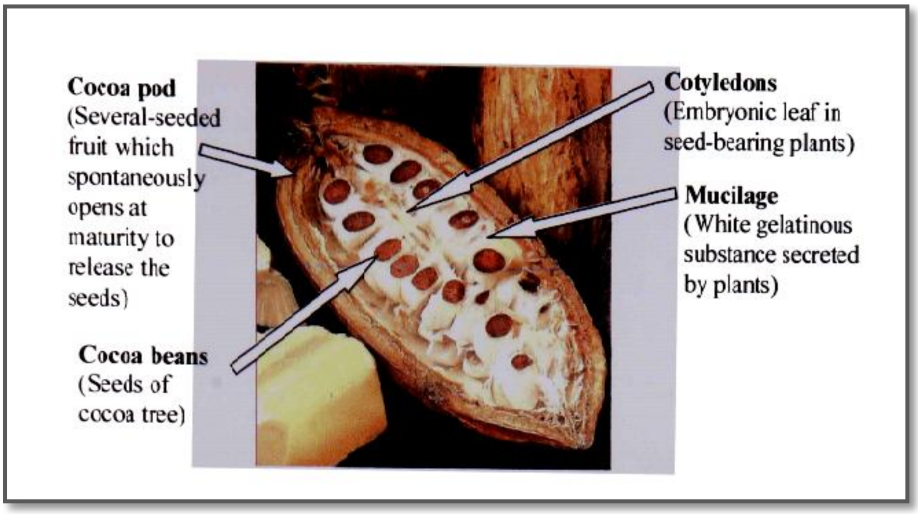

Figure 2.1 A pod with cocoa beans covered by mucilage

Ivory coast, Ghana, Indonesia, Cameroon, Nigeria, Brazil, the Dominican Republic and Malaysia are among the most important cocoa producing countries and share about 90% of the world's cocoa production (International Communications Consultancy Organization (ICCO), 2009/2010 and (FAO, 2012). The global production season of cocoa beans from 2009/2010 to 2012 were shown in Table 2.1. The most common areas of cocoa production are African countries, accounting for 69.8% of total world production, followed by Oceania and Asia with 19.8%, and the Americas (10.3%) in 2009 to 2010. While in 2012, Africa, Americas and Asian countries produced cocoa 69.1%, 12.2% and 18.8%, respectively.

Table 2.1 Dry cocoa bean production

Countries	Production ('000 tons)		Total of production (%)		
	2009/2010	2012	2009/2010	2012	2016
Africa					**72.3**
Ivory Coast	1242	1175	35.30	41.92	
Ghana	632	398	17.96	14.20	
Nigeria	240	202	6.82	7.21	
Cameroon	190	121	5.40	4.32	
Others	154	40	4.38	1.43	
America					**16.7**
Brazil	161	130	4.58	4.64	
Others	201	210	5.71	7.49	
Asia and Oceania					**11.0**
Indonesia	535	393	15.21	14.02	
Malaysia	58	79	1.65	2.82	
Papua New Guinea	50	35	1.42	1.25	
Others	55	20	1.56	0.71	
World total	3518	2803	100	100	**100**

-Source: (ICCO) International Communications Consultancy Organization, vol. xxxvi, no. 4, Cocoa year 2009/2010; FAOSTAT, 2012 and Afoakwa, 2016.

In Malaysia, cocoa production fell day by day, with cocoa output reaching 15,000 tonnes in 2011 from 35180 tonnes in 2007 (Malaysian Cocoa Board (MCB), 2011). Cocoa consumes about 71% of global production, especially in African countries, including Ivory Coast, Ghana and Nigeria (Afoakwa, 2016). In the last years, cocoa consumption has been reduced due to the gradual decline of its production (Fowler and Coutel, 2017). Generally, the Malaysian cocoa based products were exported to 270 million Malaysian Ringgit or more than returns in 2010 (Zzaman, 2015).

2.1.2 Cocoa bean properties

The chemical component and the cocoa butter characteristics are affected by the diversity of cocoa beans and cocoa growth circumstances. Therefore, there are many differences between cocoa butter varieties and there are differences within one group.

2.2 Cocoa butter

Cocoa butter acts as a persistent phase of chocolate and contributes to the non-greasy portion (Smith, 2001).This continuous phase is of great importance in influencing the properties of chocolate, which include gloss, 'snap', thermal stability, flavor and perception of the mouth feel (Norberg, 2006). Cocoa seeds are known as cocoa beans, which have the same content of the cotyledon (NIP) and shell of 85% and 15% sequentially. Cocoa beans also contain 55% fat. The paste of the crushed grains (cocoa bean paste) is called cocoa liquor or mass, which is usually used in the chocolate industry directly. Cocoa beans are natural oil seeds similar to palm kernel, peanuts, sesame seeds or any other type of oilseed. The processes performed for extracting and obtaining oil from these grains are not similar to those of other oilseeds. The process to extract and obtain the oil from these grains do not resemble those of other oilseeds due to the unparalleled physical and chemical properties of fat (Adeyeye *et al*., 2010). In general, Cocoa butter is very important in the manufacture of chocolate, by extracting from cocoa beans and cocoa mass through the process of pressing or solvent extraction (Smith, 2001).

2.2.1 Natural composition of cocoa butter

Cocoa butter is based on a 57% dry weight and in charge of the solubility characteristics of chocolate (Steinberg *et al*., 2003). Staphylakis and Gegiou. (1985) detected the level of sterols in the cocoa butter, such as methylsterols, desmethylsterols and triterpenes. In another study, they found vitamin E such as β-tocopherol, α-tocopherol and γ-tocopherol in cocoa butter, the β-tocopherol was found in higher amount followed by α-tocopherol and γ-tocopherol (Erickson *et al.*, 1983). The main triacylglycerol in cocoa butter account for 92-96% of total fat composition, respectively, these triacylglycerol include: Glycerol-1, 3-dipalmitate-2-oleate (POP); glycerol-1-palmitate-2-oleate-3-stearate (POS) and glycerol-1,3-distearate-2-oleate (SOS) (Asep *et al*., 2008; Davis and Dimick, 1989; Lehrian and Keeney, 1980; Lipp *et al*., 2001). POS is the main triglyceride found in cocoa butter at 42.5-46.4%, while the SOS is 27.8-33.0% cocoa butter, followed by POP at 18.9-22.66% respectively. (Asep *et al*., 2008). The major fatty acids of cocoa butter are palmitic acid (C16) 25–33.7%, stearic acid (C18:0) 33.7–40.2%, oleic acid (C18:1) 26.3–35% and linolenic acid (C18:3) 1.7–3%, which contribute about 98% of the total fatty acid (Asep *et al.*, 2008; Bracco, 1994; Davis and Dimick, 1989; Lipp and Anklam, 1998; Spangenberg and Dionisi, 2001; Lehrian and Keeney, 1980; Kheiri, 1982). The composition of cocoa butter differs from fatty acids according to the country of origin are shown in Figure 2.2.

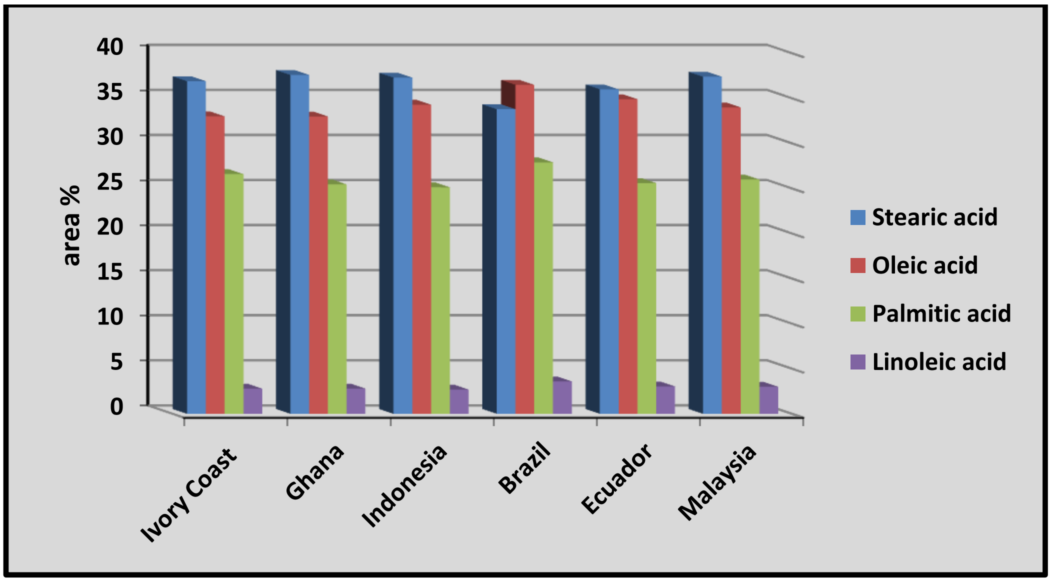

Figure 2.2 The major fatty acid composition of natural cocoa butter produced in different countries (Staphylakis and Gegiou, 1985)

2.2.2 Physicochemical properties of cocoa butter

Cocoa butter (CB) is one of the main ingredients commonly used in the manufacture of chocolate and other sweets because of its important physical and chemical properties. These characteristics of the CB are solid at room temperature (less than 25°C) and are liquid at body temperature (approximately 37°C). CB can crystallize into several polymorphic forms, having α, γ, β', and β crystals, with melting points of 17, 23, 26, 35 and 37°C respectively. β crystal is only used in the chocolate production due to its melting point is high. The crystal structure of cocoa butter makes chocolate of high quality in terms of gloss, snap and soft texture. (Kheiri, 1982).

Cocoa butter is mainly made up about 97% of the triacylglycerol, while the rest, such as free fatty acids (FFA), monoacylglycerols, diacylglycerols, phospholipids, is a minor component of 3% (Smith, 2001). The triacylglycerol consists of three fatty acids associated with the glycerol molecule. Cocoa butter is composed of three essential saturated fatty acids: palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) (20-26) %, (29-38) % and (29-38) %, respectively. Those saturated fatty acids provide the high melting point in CB due to the occurance of medium chain fatty acid and the organized structure of the CB crystal with small size crystal make CB difficult to melt and resulted slow crystal formation. Therefore, Cocoa butter dissolves rapidly over a narrow temperature due to its content of triacylglycerols (Talbot, 2009b). Cocoa butter has triacylglycerols crystallized in a higher melting fraction (mainly SOS) and a low melting fraction (mainly POP and POS) (Norberg, 2006). On the other hand, one of the chemical characteristics of cocoa butter is the iodine value (IV), have a significant impact on the efficiency of oil as it refers to the level of unstauration in CB, ultimately contributing to the softness of cocoa butter whereby higher iodine value content result in softer butter (Chaiseri and Dimick, 1989). Saponification value represents the average chain length of fatty acid fats. The higher saponification value represents short chain length fatty acid and vice versa. The acid value (AV) represents the amount of free fatty acids in 1 gram of fat was used to determine the amount of free fatty acids that are found in fat (Amin *et al*., 2002).

2.2.3 Cocoa butter for chocolate production

The fat is responsible for melting behavior and the dispersion of all other ingredients so that, it considers as continuous phase in a chocolate product. Cocoa butter has various crystal forms. A careful tempering of the chocolate is necessary in order to get the fine crystals in the correct form (β form). Without this tempering, cocoa butter tends to crystallize in rather rough crystals, with the tendency to bloom. The bloom can be defined as the occurrence of large white and fatty crystals on the surface of chocolate, which in turn is considered undesirable when they appear. An important characteristic of cocoa butter is having a large amount of 2-oleyl glycerides of palmitic and stearic acid (POP, POS, SOS). These triglycerides make cocoa butter have the desired properties of crystallization and melting properties. Therefore, they have great importance to chocolate product in terms of the sharp melting at body temperature. The behavior of melting in cocoa butter allows a great deal of cool feeling in the mouth (the typical feeling in the mouth) when eating high-quality chocolate. For this reason, when replacing cocoa butter with other fats, taken into consideration that the melting behavior should be very similar to the cocoa butter to achieve the same mouthfeel and when replacing the cocoa butter in part with other fats, the added fat should not completely change the thermal behavior of cocoa butter (M. Lipp* and E. Anklam, 1998).

2.3 Cocoa butter alternatives

The high and unstable price of cocoa butter forced confectioners to seek of cheaper and more readily available alternative fats derived from various natural sources. Cocoa butter can be replaced with other fats called cocoa butter alternatives (CBA). It is important since it contributes to an optimal melting profile thus providing a desirable texture as well as contributing to the characteristic snap upon breakage (Shukla, 2005). Cocoa butter alternatives (CBA) can be divided into different groups according to their function and similarity with cocoa butter. The first group is called cocoa butter equivalents (CBEs), like illipe butter, palm oil, shea butter, mango kernel fat and kokum butter. The second group is cocoa butter replacers (CBRs), such as palm oil, soybean oil, rape seed oil and cotton seed oil. The last group is cocoa butter substitutes (CBSs), such as palm kernel oil and coconut oil. All these groups can alternative of cocoa butter because they have economic and technological advantages that allow them to replace with other natural fats in whole or in part (Stewart and Timms, 2002; Talbot, 2009b; Norberg, 2006; Timms, 2003).

2.3.1 Properties and legislation

The European Commission has introduced new provisions allowing the replacement of cocoa butter with other vegetable fats by up to 5% European Economic Community (EEC). Therefore, the added vegetable fats should have distinct functional differences which can be described as follows (Brankmann, 1992; Boucholte, 1994).

(a) Cocoa butter equivalent (CBE): non-lauric plant fats and similar to cocoa butter in their physical and chemical properties, which are mixable with CB in every amount without changing the characteristics of it. The CBEs are subdivided into two groups as shown in Figure 2.3:

(i) Cocoa butter extender (CBEX): It is a subset of the CBE and cannot be mixed with cocoa butter in any proportion.

(ii) Cocoa butter improvers (CBIs): Similar to CB, but contains a higher ratio of solid triglycerides than CB which can be used to improve its softness. They mainly consist of palmitic acid, stearic acid, oleic acid, linoleic acid and arachidic acid in the combination of POP, POS and SOS in triglyceride, where P represents a palmitic acid; O, oleic acid and S, stearic acid (Brinkmann in Lipp, 1998; Francis *et al.*, 1999).

(b) Cocoa butter replacer (CBR): Non-lauric fat is also similar to cocoa butter in terms of fatty acids. Its structure of triglycerides is completely different from cocoa butter; only in small proportions appropriate to cocoa butter, which mainly consist of elaidic acid, stearic acid, palmitic acid and linoleic acid (Palmitic-Elaidic-Elaidic& Stearic-Elaidic-Elaidic configuration) are commonly used as CBRs (Brinkmann in Lipp, 1998).

(c) Cocoa butter substitutes (CBSs): Lauric plant fats (containing lauric acid), chemically different from cocoa butter, but having some physical similarities, however, considered suitable for substitute with cocoa butter with the medium chain triglycerides (mostly lauric and palmitic acids in configuration Lauric-Lauric-Lauric, Lauric-Lauric-Myristic and Lauric-Myristic-Myristic) (Lipp, 1998).

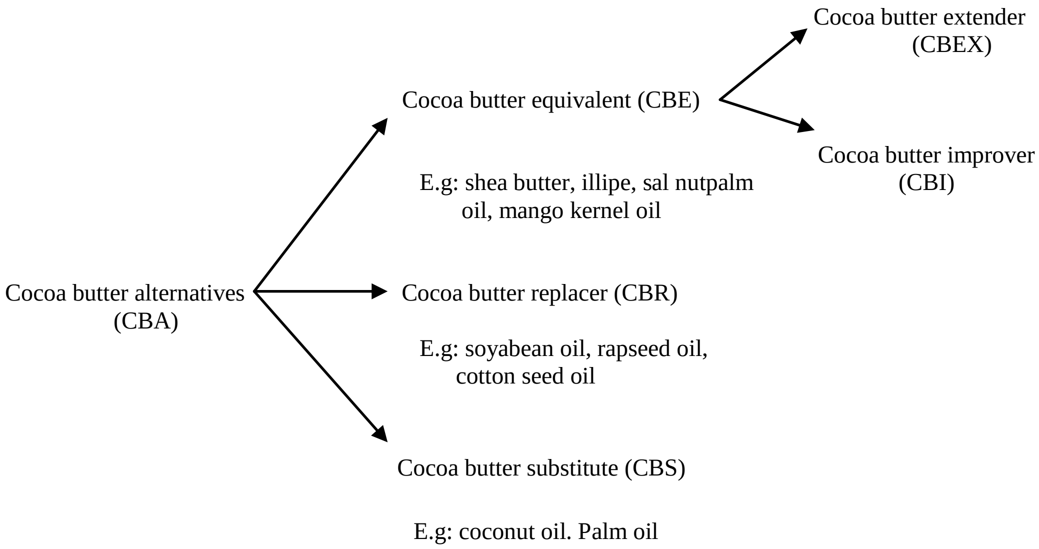

Figure 2.3 Subgroups of cocoa butter alternative (Naik and Kumar, 2014)

In some countries, vegetable fats were allowed for using as partial replacement of CB. External fats having similar chemical and physical characteristics to those of CB are normally added to chocolate. The triglyceride composition of cocoa butter can be considered as indicators of cocoa butter alternative due to it constitutes more than 95% of the cocoa butter composition (Lipp and Anklam., 1998; Zaidul *et al.*, 2006). CBRs, CBE and CBS can be produced by chemical or enzymatic fragmentation of plant fats (Lipp and Anklam, 1998; Nesaretnam and Ali, 1992; Reddy and Prabhakar, 1990).

2.3.2 Modification techniques to develop cocoa butter alternatives

Fats derived from natural sources have physical properties similar to those of cocoa butter. Subsequently, these alternatives of fat were produced by mixing or modifying fat at certain ratio (Jahurul *et al.*, 2013). The production of the CB alternatives (CBA) which involves blending, fractionation, hydrogenation, and chemical or enzymatic interesterification has gained great interest due to its negative and adverse health effects (Sundram *et al*., 2007). Most cocoa butter alternatives are prepared by blending which is mechanical mixing and one of the oldest methods of fat modification. In this process, selected fats and their fractions are mixed together to get the triacylglycerols composition, similar to that of cocoa butter (Jahurul *et al*., 2013; Stewart and Timms, 2002). While, fractionation is performed the initial fat into two or more fractions and the difference in the melting temperature values of the triacylglycerols composition in the oil (Ganesh and Rekha, 2013; Raju and Reni, 2001). In this process, the fat is melted and then cooled to get the crystals. Two main methods are used for fractionation of fats: Dry and solvent fractionation. Dry fractionation includes the selective crystallization of the high melting triglycerides followed by filtration, without solvent (Arnaud *et al*., 2006). Whereas, solvent fractionation process consists of the crystallization of a required fraction of fat, which melted in an organic solvent, usually hexane or acetone (Minifie, 1989). In another modification method is hydrogenation, which is used to change the chemical properties of lipids. This method involves adding hydrogen to the double bonds of unsaturated fatty acids. The aim of this process is to convert fat and oil into products with best physicochemical properties: best plasticity, hardest consistency (increasing the melting temperature of fat) and biggest resistance to oxidation (da Silva Lannes and Ignasio, 2013). Finally, the effective technique is interesterification which used to modify the physicochemical characteristics of oils and fats. Interesterification lead to a distribution of the fatty acids within and between the triacylglycerols. As a result of fat interesterification the structure and composition of triacylglycerols are changed, however the fatty acid composition still without changed, fatty acids are biologically active after this treatment (Afoakwa, 2008; Raju and Reni, 2001). All aforementioned modification methods are most commonly used to change the chemical, physical and sensory properties of fats (Vidhate and Singhal., 2013; Afoakwa *et al*., 2008). Non-chemical modification techniques and eco-friendly CBA have been reported, such as the use of enzymatic interesterification implemented by Bootello et al. (2012), Çiftçi *et al.*, (2009), and Shekarchizades *et al.*, (2009). The development of CBA by using a natural product, which have some similarities with cocoa butter properties and modified by blending or fractionation is still reported as promising alternatives that can be used (Calliauw *et al*., 2005; Zaidul *et*., 2007). In last years, Kaphueakngam *et al.*, (2009) produced cocoa butter equivalent by mix mango seed fat with palm oil mid-fraction. Seven mixtures of mango seed oil with palm oil in different proportions have been investigated using different techniques as the above-mentioned.

2.3.3 Fats commonly used as a source of cocoa butter alternative

One of the most common natural vegetable fats is palm oil and also illipe and shea. Besides these fats, there are other fats that are allowed by the European Commission to be used such as sal, kokum gurgi and mango kernel (Timms, 2003). In chocolate and confectionery production, vegetable oils are used to reduce cost, simplify production as well as improve the functionality level and stability of products. In addition, cocoa butter alternatives contribute with an optimized melting profile, provide a desirable texture and a suitable snap upon breakage (Shukla, 2005).

2.3.3.a Palm kernel oil

Palm kernel oil is an important oil used as an alternative to cocoa butter. Its fatty acid composition contain lauric acid at higher amount than other fatty acid such as stearic and oleic acid as compared with cocoa butter (Table 2.2). Palm kernel oil are fractionated using supercritical fluid to be used as a blending agent of the cocoa butter replacer. High quality palm oil can be a good replacement for cocoa butter because it contains high levels of solid fat and high melting point, that's make it possible to be applied as pastries products (Zaidul *et al*., 2006).

2.3.3.b Kokum kernel fat

Kokum kernel fat is obtained from kernels of an evergreen kokum tree (*Garcinia indica*), grown in several areas of India (Vidhate and Singhal, 2013; Jahurul *et al*., 2014). It is a byproduct of agro-processing industry, it contains about 40-50% fat, which has the ability to be used as cocoa butter alternative (Vidhate and Singhal, 2013). Kokum kernel fat is a hard, solid phase fat with a light-yellow color and mild odor (Raju and Reni, 2001). Kokum kernel fat is characterized by high melting temperature ranging between 38-42°C (Gunstone, 2011; Vidhate and Singhal., 2013).

The kokum fat fractionation allows a very high level of stearin fractions, which provide chocolate filling (Timms, 2003). Kokum kernel fat has the most important triglyceride compositions and physico-chemical characteristics as shown in Table 2.2 making it an excellent alternative to cocoa butter due to it improves the quality of chocolate in terms of hardness, prevents fat bloom and decrease the time of tempering (Jahurul *et al*., 2013; Stewart and Timms, 2002).

2.3.3.c Sal fat

Sal fat (*Shorea robusta*) extracted from the seed kernel which grow on Sal trees and heavily grew in Malaysia, India, Java, and Philippines. The solid fats are found in seeds about 14-18% and contain 56% of SOS triacylglycerols and also contain a large quantity of arachidic acid (Table 2.3). Gunston (2012) showed that sal fat fractionation is important to make triglycerides of CB alternative resemble triglycerides of cocoa butter, which is a valuable and important component for cocoa butter equivalents. Reddy and Prabhakar (1990) mentioned that possibility of cocoa butter extenders chains through change the sal fat and phulwara butter stearin proportions in the mixtures that appear similar to cocoa butter in physico-chemical characteristics.

2.3.3.d Shea butter

Shea butter is obtained from the shea kernel which contain around 40-55% oil. An African vegetable grown in sub-Saharan Africa, where one of the good fats to eat. According to the triglyceride composition; shea butter is used as CB substitutes in the chocolate and confectionery product (Olejide *et al.*, 2000). Shea butter needs to be fractionated to obtain a stearin fraction suitable for cocoa butter equivalent production (Bootello *et al*., 2012) due to fat contains elevated levels of the SOO, which considerably softens the oil (Table 2.2).

2.3.3.e Illipe butter

Illipe butter (Borneo tallow) is extracted from tropical tree seeds *(Shorea stenoptera*) of the family Dipterocarpaceae, illipe tree grows in the forests of Java, Borneo, the Philippines, Malaysia and Sumatra (Jahurul *et al*., 2013; Lipp and Anklam, 1998).

The ripe Illipe nuts contain 40-60% valuable edible fats (Bockisch, 1998). Illipe butter has melting point around 37 to 38 °C (Firestone, 1999). This butter is highly stable towards oxidation. The triglyceride composition of Illipe butter is closely similar to that of CB as shown in Table.2.2, therefore it's possible for using these fats directly as an equivalent of the cocoa butter (Lippe and Anklam, 1998; Gunstone, 2011; Jahurul *et al*., 2013).

2.3.3.f Mango kernel fat

The mango kernel fat is extracted from seeds of *Mangifera indica* L. The mango tree is grown in tropical countries of the world, such as India, Brazil, Mexico, Pakistan, China and Indonesia (Jahurul *et al*., 2013; 2014). The kernel consists of about (7 - 15) % fat and has melting point about (34 – 43) °C. It's fat has very important fatty acid such as palmitic, stearic and oleic acids (Abdalla, 2007; Gunstone, 2011; Maheshwari and Reddy, 2005; Nzikou *et al*., 2010; Jahurul *et al*., 2013). Depend on the triglyceride compositions (Table.2.2), mango seed fat is similar to that of natural cocoa butter and can be considered as cocoa butter equivalent (Jahurul *et al*., 2014).

Table 2.2 Chemical properties of different fats commonly used as a replacer of cocoa butter

Fatty acid (%)	Palm kernel fat	Kokum kernel fat	Sal fat	shea butter	Illipe butter	Mango kernel fat	CB
Palmitic	44.1	−	04.6-08.3	03.4-08.0	18-21	03-18	25.2-33.7
Stearic	04.0	50-60	34.7-43.2	37.0-58.0	39-46	24-57	33.3-40.2
Oleic	39.0	36-40	40.4-42.4	33.0-50.0	34-37	34-56	26.3-35.2
Linoleic	10.0	−	01.5-02.8	03.0-06.6	−	01-13	01.7-03.6
Arachidic	0.3	−	06.1-12.3	0.2-02.0	−	01-04	−
Triglycerides							
POP	26	Trace	−	3	7	1	18.9-23.4
SOS	−	72	42	42	45	40-59	27.5-33.0
POS	3	6	11	6	34	11-16	42.8
SOO	3.3	−	16	−	26	23	−
SOL	−	−	−	−	5	−	−
SLS	−	−	−	−	5	−	−
OOO	4.6	−	3	−	6	5	−
AOO	−	−	4	−	−	−	−
SOA	−	−	13	−	−	4	−
POO	27.5	−	−	−	−	5	−

-Source: (Gunstone, 2011**)**. POP: (palmitic-oleic-palmitic), SOS: (stearic-oleic-stearic), POS: (palmitic-oleic-stearic), SOO: (stearic-oleic-oleic), SOL: (stearic-oleic-linolenic), SLS: (stearic-linolenic- stearic), OOO: (oleic- oleic- oleic), AOO: (arachidic oleic- oleic), SOA: (stearic-oleic- arachidic), POO: (palmitic- oleic-oleic).

2.3.3 (g) Other fats

In the past, other fats have been successfully used as cocoa butter alternatives, such as aceituno oil and dhupa fat (Timms, 2003). It has been noted at present that it is rare to obtain natural fats from vegetable sources and therefore it is important to find new sources, some of them non-traditional such as agroindustrial waste. In recent years, there has been an obvious tendency in favor of used fats from natural sources such as Cupuassu fat (Silva *et al*., 2009), tea seed oil (Soheila *et al*., 2012), pumpkin oil (Vujasinovic *et al*., 2012), pine nut oil (Cai *et al*., 2013) and *rambutan* seed fat (Febrianto *et al*., 2014) and mix it with cocoa butter in whole or in part. These fats are hydrogenated vegetable fats and inter-sterified oil. The properties of these fats are very important in terms of industry (Solís-Fuentes *et al*., 2010).

2.4 Rambutan

2.4.1 Origin and distribution of rambutan

Rambutan (*Nephelium lappaceum* L.) is from the family Sapindaceae which is closely related to lychee (*Litchi chi-nensis Sonn.*), longan (*Dimocarpus longan Lour*.) and 'pulasan' *(Nephelium mutabile Blume*) (Marisa, 2006). *N. lappaceum* is a tropical type common to Southeast Asia, but in present time cultivation has been extended to China, India, Thailand, Taiwan, Malaysia, and Australia (Davidson *et al*., 2006; Jalikop, 2012). In Malaysia, rambutan spread cultivation in most part of the country, but is concentrated principally in the states of Perak, Pahang, Kedah, Kelantan, Johor and Terengganu. The total acreage of rambutan plantations in Peninsular Malaysia in 1985 was estimated at 16,925 ha, but was increased in 1990 to 24,341 ha and in 2000 to 49,730 ha (Malaysian Agriculture Directory and Index, 1986). Rambutan in Malay means hairy and often called 'hairy litchi' and known as 'usan', 'usau' or 'usare' in the Philippines, in Thailand 'ngo' or 'phruan', and in Cambodia 'ser mon' or 'chle sao mao' (Tindall, 1994), it is an important nectar source for bees in Malaysia (Phoon, 1983). The rambutan plant lives in a warm tropical climate at 22-30°C and is sensitive to the temperatures less than 10°C. (Morton, 1987). Trees begin flowering from March to May and August to October. The fruit matures from 15 to 18 weeks after flowering (Tindall, 1994). The skin of the rambutan fruit can differ in color from pink to deep crimson and from yellow to yellow or orange (Watson, 1988). The fruit is circular to oval in shape. The aril is sweet and its color is translucent in some varieties of rambutan it sticks to the seed, one of the most popular varieties in the market are the "freestone" variety. Seed color is usually light brown, where these seeds contain a high percentage of fat that is composed of fatty acids such as, oleic acid and archidic acid. Rambutan root bark, and leaves are used in the field of the production of dyes or in the field of medicine.

In Malaysia, rambutan cultivars are named with code letter ‘R’, and R3, R4, R99, R169, R170, RS6, and R191 (yield approximately 1.2 - 15 tons ha^{-1} $year^{-1}$) are the cultivar clones recommended by the Department of Agriculture (DOA, 2013). Whereas, according to the Indonesian Ministry of Research and Technology, there are 22 popular cultivars of rambutan, in which Binjai, Rapiah, Lebak Bulus, Antalagi, Sibongkok, Sibatuk Ganal, Garuda and Nona are cultivars that have relatively high economic value. It is also reported that the productivity of these cultivars are 40-68, 18-30, 50-100, 160-210, 175-225, 240-280, 200-270, and 20-22.5 kg $tree^{-1}$ $year^{-1}$, respectively (Ristek, 2000; Rukmana and Yuniarsih., 2002). In the latest reports of rambutan production recorded in 2011, Indonesia was reported to produce 811,909 tonne of rambutan, Vietnam 500-650 tonne, Thailand 700,000 tonne, Malaysia 86,085 tonne, Philippines 6,270 tonne. On the other hand, non-asian countries such as Mexico and Australia have been producing rambutan with the production worth 7,000 tons and 1000 tons in 2011, respectively (Survey Pertanian Produksi buah-buahan, 2013; Food and Agriculture Organization, 2007). Generally, there is a rise in demand to buy rambutan from top importers such as the United Arab Emirates, Korea and the Netherlands, and the increasing export range of the United States, and European states (Fraire, 2001; ITFN, 2013; DAFF, 2013; VietNamNet, 2013).